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# Additional chromosome abnormalities in patients with acute promyelocytic leukemia treated with all-*trans* retinoic acid and chemotherapy

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## ABSTRACT

### Background

Acute promyelocytic leukemia is a subtype of acute myeloid leukemia characterized by the t(15;17). The incidence and prognostic significance of additional chromosomal abnormalities in acute promyelocytic leukemia is still a controversial matter.

### Design and Methods

Based on cytogenetic data available for 495 patients with acute promyelocytic leukemia enrolled in two consecutive PETHEMA trials (LPA96 and LPA99), we analyzed the incidence, characteristics, and outcome of patients with acute promyelocytic leukemia with and without additional chromosomal abnormalities who had been treated with all-*trans* retinoic acid plus anthracycline monochemotherapy for induction and consolidation.

### Results

Additional chromosomal abnormalities were observed in 140 patients (28%). Trisomy 8 was the most frequent abnormality (36%), followed by abn(7q) (5%). Patients with additional chromosomal abnormalities more frequently had coagulopathy ( $P=0.03$ ), lower platelet counts ( $P=0.02$ ), and higher relapse-risk scores ( $P=0.02$ ) than their counterparts without additional abnormalities. No significant association with *FLT3*/TTD or other clinicopathological characteristics was demonstrated. Patients with and without additional chromosomal abnormalities had similar complete remission rates (90% and 91%, respectively). Univariate analysis showed that additional chromosomal abnormalities were associated with a lower relapse-free survival in the LPA99 trial ( $P=0.04$ ), but not in the LPA96 trial. However, neither additional chromosomal abnormalities overall nor any specific abnormality was identified as an independent risk factor for relapse in multivariate analysis.

### Conclusions

The lack of independent prognostic value of additional chromosomal abnormalities in acute promyelocytic leukemia does not support the use of alternative therapeutic strategies when such abnormalities are found.

**Key words:** acute promyelocytic leukemia, additional chromosomal abnormalities, prognostic factors, all-*trans* retinoic acid, anthracycline.

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## Introduction

Cytogenetics is the most powerful single prognostic factor for outcome in acute myeloid leukemia<sup>1-3</sup> and the most useful guide available for stratification and planning post-remission treatment in this disease. The t(15;17), characterizing the acute promyelocytic form of acute myeloid leukemia, is considered to be a favorable cytogenetic feature. However, the prognostic significance of additional cytogenetic abnormalities (ACA) in acute promyelocytic leukemia (APL) has remained a matter of debate.

During the 1990s, some studies suggested a relationship between ACA and outcome in APL.<sup>4,5</sup> However, these studies were retrospective and performed in small series of patients mostly treated with chemotherapy alone. More recently, three studies undertaken in patients with APL managed with state-of-the-art treatments, that is, a simultaneous combination of all-*trans* retinoic acid (ATRA) with anthracycline-based chemotherapy, have yielded conflicting results with regard to the impact of ACA on prognosis. In two large studies ACA were not found to have an impact on prognosis,<sup>6,7</sup> while, in the third study, patients with ACA had a higher death rate during induction therapy compared with patients exhibiting the t(15;17) alone.<sup>8</sup> Although none of these studies demonstrated that ACA in APL have a significant impact on the risk of relapse, physicians may be tempted to modify the planned treatment based on the presence of these abnormalities, extrapolating strategies used for the management of other subtypes of acute myeloid leukemia.

In order to clarify the role of ACA in APL patients treated with modern treatments, we report here the characteristics, outcome and prognostic value of cytogenetics in a large cohort of successfully karyotyped patients with a long follow-up who were enrolled in two successive studies carried out by the Spanish *Programa de Estudio y Tratamiento de las Hemopatías Malignas* (PETHEMA) group (studies LPA96 and LPA99).

## Design and Methods

### Patients and eligibility

Between November 1996 and June 2005, a total of 739 patients with *de novo*, genetically confirmed APL were enrolled into two consecutive trials, LPA96 and LPA99. The eligibility criteria and protocols of these studies have been reported elsewhere.<sup>9-11</sup> Informed consent to participation in the studies was obtained from all patients, in accordance with the Declaration of Helsinki. The protocol was approved by the Research Ethics Board of each participating hospital.

### Diagnosis

In addition to the morphological and cytochemical criteria used by the French-American-British classification and routine immunophenotyping, the diagnosis of APL was genetically confirmed in all cases by demonstration of the *PML/RARA* hybrid gene and/or the chromosomal translocation t(15;17)(q22;q21). Immunophenotypic and cytogenetic analyses were systematically performed at presentation only. For the purpose of rapid diagnosis, an immunohistochemical analysis of PML protein distribution was performed, using the monoclonal antibody PG-M3,<sup>12</sup> in a subgroup of patients.

### Cytogenetics and fluorescence in situ hybridization

Bone marrow samples for cytogenetic analysis were processed after short-term culture (24 or 48 h) following standard procedures. The chromosomes were stained by G-banding and the karyotypes reported according to International System for Human Cytogenetic Nomenclature (ISCN, 1995) recommendations.<sup>13</sup> Whenever possible at least 20 metaphases were analyzed in each case. Cases were considered normal diploid if no clonal abnormalities were detected in a minimum of 20 mitotic cells. In most of the patients with apparently normal karyotype and *PML/RARA* rearrangement demonstrated by reverse transcriptase-polymerase chain reaction (RT-PCR), fluorescence *in situ* hybridization (FISH) studies were additionally carried out in metaphase and interphase nuclei. Two-color FISH was performed using a *PML/RARA* translocation probe (Abbott, Wiesbaden, Germany).

The majority of cytogenetic analyses were performed at reference laboratories. The original cytogenetics reports were requested from the centers for central review. Appropriate karyotype nomenclature (ISCN 1995) was centrally reviewed by two of the authors (JC, JMH). For the purposes of this study, patients with a normal karyotype with the *PML/RARA* fusion demonstrated by either RT-PCR or FISH, were considered as having APL without ACA.<sup>4,14</sup>

### Reverse transcriptase-polymerase chain reaction studies

The details on processing bone marrow samples for RNA extraction and on the RT-PCR protocols for *PML/RARA* amplification used by the participating laboratories have been described elsewhere.<sup>15,16</sup>

### Treatment

The induction regimen consisted of oral ATRA (45 mg/m<sup>2</sup>/day), divided into two daily doses, which was maintained until complete remission, and intravenous idarubicin (12 mg/m<sup>2</sup>/day) on days 2, 4, 6, and 8. For patients 20 years of age or younger, the ATRA dose was adjusted to 25 mg/m<sup>2</sup>/day. From November 1999, the idarubicin on day 8 was omitted for patients older than 70 years. Patients in complete remission received three monthly consolidation courses. The first course consisted of idarubicin (5 mg/m<sup>2</sup>/day for 4 days), the second of mitoxantrone (10 mg/m<sup>2</sup>/day for 5 days), and the third of idarubicin (12 mg/m<sup>2</sup>/day for 1 day). From November 1999 (LPA99 study), intermediate- and high-risk patients, as previously defined,<sup>17</sup> received ATRA (45 mg/m<sup>2</sup>/day for 15 days) combined with the three chemotherapy courses;<sup>10,11</sup> those based on idarubicin were slightly reinforced by increasing the dose in the first course to 7 mg/m<sup>2</sup>/day and by administering idarubicin for two consecutive days instead of one in the third course. Patients who tested negative for *PML/RARA* at the end of consolidation were started on maintenance therapy with oral mercaptopurine (50 mg/m<sup>2</sup>/day), intramuscular methotrexate (15 mg/m<sup>2</sup>/week), and oral ATRA (45 mg/m<sup>2</sup>/day for 15 days every 3 months) over 2 years. Details of the supportive therapy have been described elsewhere.<sup>9,18</sup>

### Definitions and study end-points

Response to the remission induction therapy was assessed according to criteria recently revised by Cheson *et al.*<sup>19</sup> Molecular remission was defined as the disappearance on an ethidium bromide gel of the *PML/RARA*-specific band visualized at diagnosis, using an RT-PCR assay with a sensitivity level of 10<sup>-4</sup>. Molecular

persistence was defined as PCR positivity in two consecutive bone marrow samples collected at the end of consolidation therapy. Molecular relapse was defined as the reappearance of PCR-positivity in two consecutive bone marrow samples at any time after consolidation therapy. Risk of relapse was established at diagnosis according to a predictive model based on each patient's leukocyte and platelet counts at diagnosis, as reported elsewhere.<sup>17</sup> Low-risk patients had a white cell count less than  $10 \times 10^9/L$  and a platelet count more than  $40 \times 10^9/L$ ; intermediate-risk patients had a white cell count less than  $10 \times 10^9/L$  and a platelet count less than  $40 \times 10^9/L$ ; and high-risk patients had a white cell count equal to or more than  $10 \times 10^9/L$ . The presence of coagulopathy was defined as a prolonged prothrombin time and/or activated partial thromboplastin time, in addition to hypofibrinogenemia and/or increased levels of fibrin degradation products or D-dimers.

### Statistical analysis

Differences in the distribution of variables among subsets of patients were analyzed using  $\chi^2$  and Fisher's exact tests. Unadjusted time-to-event analyses were performed using the Kaplan-Meier estimate,<sup>20</sup> and, for comparisons, log-rank tests.<sup>21</sup> For all estimates in which the event "relapse" was considered as an end-point, hematologic and molecular relapse, as well as molecular persistence (*PML/RARA*-positive by RT-PCR at the end of consolidation), were each considered as uncensored events. The follow-up of the patients was updated on January 15, 2009. The median follow-up of surviving patients was 85 months (range, 42 to 145 months). Multivariate analysis was performed using the Cox proportional hazards model.<sup>22</sup> All computations were carried out using 3D, 4F, 1L and 2L programs from the BMDP statistical library (BMDP Statistical Software Inc, Los Angeles, CA, USA).

## Results

### Incidence and characteristics of chromosomal abnormalities

Between November 1996 and June 2005, a total of 739 patients with *de novo*, genetically confirmed APL were enrolled into the consecutive LPA96 and LPA99 trials from 82 institutions in Spain, The Netherlands, Belgium, Argentina, Uruguay, and the Czech Republic (see Appendix). Cytogenetic data were not available for 244 cases because cytogenetic studies had not been performed ( $n=32$ ) or had failed ( $n=191$ ), or for unknown reasons. Failures in cytogenetic analysis were due to the absence of metaphases ( $n=124$ ) or either poor quality or insufficient number of metaphases ( $n=67$ ). All these patients were genetically diagnosed by FISH, RT-PCR or anti-PML staining. Among the remaining 495 patients (67%), 355 (72%) had the *t*(15;17) translocation as the sole chromosomal abnormality and 140 patients (28%) had ACA; 95 of the patients had one additional abnormality (67%) and 45 had two or more abnormalities (33%) (Table 1). Trisomy 8 ( $n=51$ ), either alone ( $n=37$ ) or associated with other aberrations ( $n=14$ ), was the most frequent abnormality (36%), followed by other less frequent numerical and structural aberrations listed in Table 1.

Dual color FISH studies, RT-PCR or both showed the *PML/RARA* fusion gene in the 71 patients with available karyotype in whom the *t*(15;17) was not detected by con-

ventional cytogenetics. In 45 of these patients the karyotype was normal while in the remaining 26 cases the karyotype showed other cytogenetic changes but not the *t*(15;17) (Table 1).

### Cytogenetic abnormalities and disease characteristics

The main clinical and biological characteristics of patients without an available karyotype and those with either *t*(15;17) alone or *t*(15;17) with ACA are shown in Table 2. Patients with ACA had significantly lower platelet counts ( $P=0.02$ ) and were, therefore, less frequently classified as at low-risk ( $P=0.02$ ) compared with those without ACA. A similar association with platelet counts and relapse-risk score was also observed according to the number of ACA. Patients with two or more ACA had significantly lower platelet counts ( $P=0.02$ ) and were classified less frequently as being at low-risk ( $P=0.02$ ) compared with those with a single additional chromosomal abnormality. In addition, patients with ACA more frequently had coagulopathy ( $P=0.03$ ) and, although the differences were not statistically significant, tended to be younger ( $P=0.05$ ) and more frequently had the BCR3 *PML/RARA* isoform than patients with *t*(15;17) alone ( $P=0.08$ ). The presence of trisomy 8 was significantly associated with more fever at diagnosis ( $P=0.01$ ), coagulopathy ( $P=0.02$ ), fibrinogen levels below 170 mg/dL ( $P=0.02$ ), male gender ( $P=0.05$ ), serum uric acid levels above 7 mg/dL ( $P=0.02$ ), and greater than 70% bone marrow blasts ( $P=0.03$ ), and

**Table 1.** Additional chromosomal abnormalities in patients with APL.

| Number and type of abnormality    | Conventional <i>t</i> (15;17) (%) | Cryptic <i>t</i> (15;17) (%) | Total n. of patients (%) | 5-year RFS (%) | P    |
|-----------------------------------|-----------------------------------|------------------------------|--------------------------|----------------|------|
| N. of patients                    | 424 (100)                         | 71 (100)                     | 495 (100)                | 88             |      |
| N. of chromosomal abnormalities   |                                   |                              |                          |                |      |
| Normal karyotype                  | 0 (0)                             | 45 (63)                      | 45 (9)                   | 93             | 0.34 |
| <i>t</i> (15;17) alone            | 310 (73)                          | 0 (0)                        | 310 (63)                 | 89             |      |
| One                               | 82 (19)                           | 13 (18)                      | 95 (19)                  | 86             |      |
| Two                               | 20 (5)                            | 5 (7)                        | 25 (5)                   | 83             |      |
| Three or more                     | 17 (4)                            | 3 (4)                        | 20 (4)                   | 78             |      |
| Numerical abnormalities           | 65 (15)                           | 11 (15)                      | 76 (15)                  |                |      |
| Trisomy 8                         | 44 (10)                           | 7 (10)                       | 51 (10)                  |                |      |
| Trisomy 8 alone                   | 32 (7)                            | 5 (7)                        | 37 (7)                   |                |      |
| Trisomy 8 + other                 | 12 (3)                            | 2 (3)                        | 14 (3)                   |                |      |
| Other numerical                   | 21 (5)                            | 4 (6)                        | 25 (5)                   |                |      |
| Structural abnormalities          | 54 (13)                           | 10 (14)                      | 64 (13)                  |                |      |
| Abn(7q)                           | 6 (1)                             | 1 (1)                        | 7 (1)                    |                |      |
| Abn(9q)                           | 5 (1)                             | 0 (0)                        | 5 (1)                    |                |      |
| Abn(1p)                           | 4 (1)                             | 1 (1)                        | 5 (1)                    |                |      |
| Abn(11q)                          | 5 (1)                             | 0 (0)                        | 5 (1)                    |                |      |
| Abn(3q)                           | 3 (1)                             | 1 (1)                        | 4 (1)                    |                |      |
| i(17q)                            | 3 (1)                             | 1 (1)                        | 4 (1)                    |                |      |
| Abn(20q)                          | 4 (1)                             | 0 (0)                        | 4 (1)                    |                |      |
| Complex variant <i>t</i> (15;17)* | 0 (0)                             | 4 (6)                        | 4 (1)                    |                |      |
| Other structural                  | 25 (6)                            | 1 (1)                        | 26 (5)                   |                |      |

\* complex variant *t*(15;17) due to a 3-way balanced translocation involving 15q22, 17q21, and another chromosome. RFS: relapse-free survival.

tended to be associated with lower platelet counts ( $P=0.07$ ). The clinicopathological characteristics of patients with trisomy 8 alone did not differ from those of patients with trisomy 8 plus other abnormalities.

### ***Influence of additional cytogenetic abnormalities on outcome in acute promyelocytic leukemia***

Three hundred and twenty-five of the 355 patients (91%) with the  $t(15;17)$  alone and 126 of the 140 (90%) with ACA achieved complete remission. These rates were

**Table 2.** Demographic and baseline characteristics of the study population.

| Characteristic                  | Non available cytogenetics<br>Median<br>(range) | N. (%)    | P <sup>a</sup> | t(15;17) alone<br>Median<br>(range) | N. (%)    | t(15;17) with other abnormalities<br>Median<br>(range) | N. (%)    | P <sup>@</sup> |
|---------------------------------|---|-----------|----------------|-------------------------------------|-----------|--|-----------|----------------|
| Overall                         |   | 244 (100) |                |                                     | 355 (100) |  | 140 (100) |                |
| Age, years                      | 40 (2-81)                                       |           | 0.13           | 41 (2-83)                           |           | 39 (3-73)  |           | 0.05           |
| 18 or younger                   |   | 35 (14)   |                |                                     | 31 (9)    |  | 18 (13)   |                |
| 19-50                           |   | 139 (57)  |                |                                     | 207 (58)  |  | 83 (59)   |                |
| 51-60                           |   | 32 (13)   |                |                                     | 41 (12)   |  | 22 (16)   |                |
| 61-70                           |   | 26 (11)   |                |                                     | 47 (13)   |  | 14 (10)   |                |
| 71 or older                     |   | 12 (5)    |                |                                     | 29 (8)    |  | 3 (2)     |                |
| Gender                          |   |           | 0.69           |                                     |           |  |           | 0.37           |
| Male                            |   | 121 (50)  |                |                                     | 177 (50)  |  | 76 (54)   |                |
| Female                          |   | 123 (50)  |                |                                     | 178 (50)  |  | 64 (46)   |                |
| ECOG score                      |   |           | 0.69           |                                     |           |  |           | 0.84           |
| 0-1                             |   | 172 (76)  |                |                                     | 245 (76)  |  | 95 (75)   |                |
| 2-3                             |   | 54 (24)   |                |                                     | 84 (24)   |  | 31 (25)   |                |
| WBC count, $\times 10^9/L$      | 2.4 (0.3-164)                                   |           | 0.21           | 2.0 (0.2-460)                       |           | 2.8 (0.3-210)  |           | 0.17           |
| Less than or equal to 3.5       |   | 143 (59)  |                |                                     | 229 (64)  |  | 75 (54)   |                |
| 3.5-10                          |   | 34 (14)   |                |                                     | 43 (12)   |  | 29 (21)   |                |
| 10-50                           |   | 54 (22)   |                |                                     | 65 (18)   |  | 33 (23)   |                |
| Higher than 50                  |   | 12 (5)    |                |                                     | 18 (4)    |  | 3 (2)     |                |
| Platelet count, $\times 10^9/L$ | 20 (1-207)                                      |           | 0.32           | 23 (1-207)                          |           | 20 (1-137)   |           | 0.02           |
| Less than or equal to 40        |   | 191 (79)  |                |                                     | 261 (73)  |  | 116 (83)  |                |
| Higher than 40                  |   | 52 (21)   |                |                                     | 94 (27)   |  | 24 (17)   |                |
| Creatinine, mg/dL               |   |           | 0.96           |                                     |           |  |           | 0.99           |
| Less than or equal to 1.4       |   | 231 (98)  |                |                                     | 338 (98)  |  | 133 (98)  |                |
| Higher than 1.4                 |   | 4 (2)     |                |                                     | 7 (2)     |  | 3 (2)     |                |
| Coagulopathy                    |   |           | 0.66           |                                     |           |  |           | 0.03           |
| No                              |   | 54 (22)   |                |                                     | 93 (26)   |  | 24 (17)   |                |
| Yes                             |   | 188 (78)  |                |                                     | 259 (74)  |  | 116 (83)  |                |
| Fibrinogen, mg/dL               |   |           | 0.51           |                                     |           |  |           | 0.76           |
| Less than or equal to 170       |   | 132 (57)  |                |                                     | 179 (55)  |  | 72 (53)   |                |
| Higher than 170                 |   | 99 (43)   |                |                                     | 147 (45)  |  | 63 (47)   |                |
| Albumin, g/dL                   |   |           | 0.83           |                                     |           |  |           | 0.43           |
| Less than or equal to 3.5       |   | 44 (22)   |                |                                     | 65 (23)   |  | 22 (19)   |                |
| Higher than 3.5                 |   | 152 (78)  |                |                                     | 221 (77)  |  | 93 (81)   |                |
| Morphologic subtype             |   |           | 0.89           |                                     |           |  |           | 0.92           |
| Hypergranular                   |   | 197 (82)  |                |                                     | 288 (82)  |  | 113 (82)  |                |
| Microgranular                   |   | 44 (18)   |                |                                     | 62 (18)   |  | 25 (18)   |                |
| PML/RARA isoform                |   |           | 0.47           |                                     |           |  |           | 0.08           |
| BCR1/BCR2                       |   | 132 (60)  |                |                                     | 186 (60)  |  | 64 (51)   |                |
| BCR3                            |   | 87 (40)   |                |                                     | 124 (40)  |  | 62 (49)   |                |
| Relapse-risk group              |   |           | 0.66           |                                     |           |  |           | 0.02           |
| Low                             |   | 45 (19)   |                |                                     | 80 (23)   |  | 16 (11)   |                |
| Intermediate                    |   | 132 (56)  |                |                                     | 192 (54)  |  | 88 (63)   |                |
| High                            |   | 66 (25)   |                |                                     | 83 (23)   |  | 36 (26)   |                |
| FLT3/ITD                        |   |           | 0.34           |                                     |           |  |           | 0.18           |
| Yes                             |   | 22 (27)   |                |                                     | 33 (23)   |  | 12 (19)   |                |
| No                              |   | 60 (73)   |                |                                     | 112 (77)  |  | 51 (81)   |                |
| Protocol                        |   |           | 0.49           |                                     |           |  |           | 0.52           |
| LPA96                           |   | 54 (22)   |                |                                     | 84 (24)   |  | 37 (26)   |                |
| LPA99                           |   | 190 (78)  |                |                                     | 271 (76)  |  | 103 (74)  |                |

<sup>a</sup>P values of the comparison of patients without evaluable karyotype versus patients successfully karyotyped. <sup>@</sup>P values of the comparison of patients with  $t(15;17)$  alone versus patients with  $t(15;17)$  and additional abnormalities.



not statistically different (Table 3). The complete remission rate among patients for whom cytogenetic data were unavailable or inadequate was not different (89%,  $P=0.26$ ).

Concerning the subsequent clinical outcome of patients who achieved complete remission, a total of 53 relapses were recorded (34 clinical and 19 molecular relapses, including five with molecular disease persistence after consolidation therapy). The overall 5-year relapse-free survival, disease-free survival, and overall survival rates were 88%, 85%, and 83%, respectively. The corresponding rates among patients for whom cytogenetic data were unavailable or inadequate were 85%, 80%, and 76% ( $P=0.28$ ,  $P=0.12$ , and  $P=0.08$ , respectively).

The results of univariate analysis of relapse-free survival are presented in Table 4. In patients with available karyotype, when both protocols LPA96 and LPA99 were considered together, several variables, such as gender, relapse-risk score, morphological subtype, and *PML/RARA* isoform, had a statistically significant prognostic value, but the presence of ACA did not ( $P=0.10$ ). When analyzed separately, trisomy 8 was associated with a statistically lower relapse-free survival compared with the absence of trisomy 8 (78% versus 89%,  $P=0.03$ ). The relapse-free survival was lower in relation to the number of chromosomal abnormalities detected by conventional karyotyping, but the differences were not statistically significant (5-year relapse-free survival of 93% in patients with a normal karyotype; 89% in patients with *t*(15;17) alone; 86% in those with one ACA; 83% with two ACA; and 78% with three or more

ACA;  $P=0.34$ ) (Table 1). Multivariate analysis identified relapse-risk score and male gender as the only independent adverse factors for relapse-free survival ( $P<0.0001$  and  $P=0.03$ , respectively).

Given the better outcome of the patients treated in the LPA99 trial compared with those in LPA96, as reported in previous analyses of this series when patients with and without available karyotype were included,<sup>10,11</sup> we performed an analysis separately by protocol (Table 4). The

**Table 3. Complete remission, overall survival, disease-free survival and relapse-free survival rates in patients with and without additional chromosome abnormalities.**

|                                   | <b>t(15;17) alone</b> | <b>t(15;17) with other abnormalities</b> |      |
|-----------------------------------|-----------------------|--|------|
| Outcome                           | (%)                   | (%)                                      | P    |
| <b>LPA96 &amp; LPA99 patients</b> |                       |  |      |
| Complete remission                | 91                    | 90                                       | 0.59 |
| 5-year overall survival           | 84                    | 81                                       | 0.82 |
| 5-year disease-free survival      | 86                    | 82                                       | 0.42 |
| 5-year relapse-free survival      | 90                    | 84                                       | 0.10 |
| <b>LPA96 patients</b>             |                       |  |      |
| 5-year relapse-free survival      | 81                    | 89                                       | 0.33 |
| <b>LPA99 patients</b>             |                       |  |      |
| 5-year relapse-free survival      | 92                    | 82                                       | 0.01 |

**Table 4. Univariate and multivariate analysis for relapse-free survival in the study population.**

| Characteristic            | LPA96 & LPA99 trials (n=451)     |        |                   |                          | LPA96 trial (n=108)              |        |                   |                          | LPA99 trial (n=343)              |        |                   |                          |
|---------------------------|----------------------------------|--------|-------------------|--------------------------|----------------------------------|--------|-------------------|--------------------------|----------------------------------|--------|-------------------|--------------------------|
|                           | Univariate<br>5-years<br>RFS (%) | P      | Multivariate<br>P | Hazard ratio<br>[95% CI] | Univariate<br>5-years<br>RFS (%) | P      | Multivariate<br>P | Hazard ratio<br>[95% CI] | Univariate<br>5-years<br>RFS (%) | P      | Multivariate<br>P | Hazard ratio<br>[95% CI] |
| Overall                   | 88                               |        |                   |                          | 84                               |        |                   |                          | 90                               |        |                   |                          |
| Gender                    |                                  |        |                   |                          |                                  |        |                   |                          |                                  |        |                   |                          |
| Male                      | 85                               | 0.02   | 0.02              | 0.59<br>[0.38-0.92]      | 83                               | 0.79   | NS                |                          | 85                               | 0.01   | 0.01              | 0.46<br>[0.27-0.80]      |
| Female                    | 92                               |        |                   |                          | 85                               |        |                   |                          | 93                               |        |                   |                          |
| Relapse-risk group*       |                                  |        |                   |                          |                                  |        |                   |                          |                                  |        |                   |                          |
| Low risk                  | 95                               | <0.001 | <0.001            | 3.60<br>[2.48-5.22]      | 91                               | 0.01   | 0.01              | 2.67<br>[1.23-5.79]      | 96                               | <0.001 | <0.001            | 3.49<br>[2.16-5.65]      |
| Intermediate risk         | 91                               |        |                   |                          | 88                               |        |                   |                          | 92                               |        |                   |                          |
| High risk                 | 74                               |        |                   |                          | 65                               |        |                   |                          | 77                               |        |                   |                          |
| Cytogenetics              |                                  |        |                   |                          |                                  |        |                   |                          |                                  |        |                   |                          |
| t(15;17)                  | 90                               | 0.10   | NS                |                          | 81                               | 0.33   | NS                |                          | 92                               | 0.01   | NS                |                          |
| t(15;17) + other          | 84                               |        |                   |                          | 89                               |        |                   |                          | 82                               |        |                   |                          |
| Morphological subtype     |                                  |        |                   |                          |                                  |        |                   |                          |                                  |        |                   |                          |
| Hypergranular             | 90                               | 0.02   | NS                |                          | 89                               | <0.001 | NS                |                          | 90                               | 0.60   | NS                |                          |
| Microgranular             | 81                               |        |                   |                          | 56                               |        |                   |                          | 88                               |        |                   |                          |
| <i>PML/RARA</i> α isoform |                                  |        |                   |                          |                                  |        |                   |                          |                                  |        |                   |                          |
| BCR1/BCR2                 | 90                               | 0.02   | NS                |                          | 83                               | 0.89   | NS                |                          | 92                               | 0.004  | 0.02              | 1.85<br>[1.02-3.33]      |
| BCR3                      | 82                               |        |                   |                          | 82                               |        |                   |                          | 82                               |        |                   |                          |
| PETHEMA LPA trial         |                                  |        |                   |                          |                                  |        |                   |                          |                                  |        |                   |                          |
| LPA96                     | 84                               | 0.09   | NS                |                          | NA                               | NA     |                   |                          | NA                               | NA     |                   |                          |
| LPA99                     | 90                               |        |                   |                          | NA                               |        |                   |                          |                                  |        |                   |                          |

RFS: relapse-free survival; NA: not applicable; NS: not significant. \*Low risk: WBC  $\leq 10 \times 10^9/L$  and platelets  $>40 \times 10^9/L$ ; intermediate risk: WBC  $\leq 10 \times 10^9/L$  and platelets  $\leq 40 \times 10^9/L$ ; high risk: WBC  $>10 \times 10^9/L$ .

univariate analysis showed that the presence of ACA at diagnosis was significantly associated with lower relapse-free survival in the LPA99 trial (82% versus 92%,  $P=0.01$ ) (Figure 1A), but not in the LPA96 trial (89% versus 81%,  $P=0.33$ ). In the LPA99 trial, univariate analysis also showed a lower relapse-free survival for patients with trisomy 8 (77% versus 91%,  $P=0.02$ ) (Figure 1B). Multivariate analysis showed that in addition to relapse-risk score and male gender, the BCR3 isoform was an independent adverse factor for relapse-free survival in the LPA99 trial, but the presence of ACA was not.

## Discussion

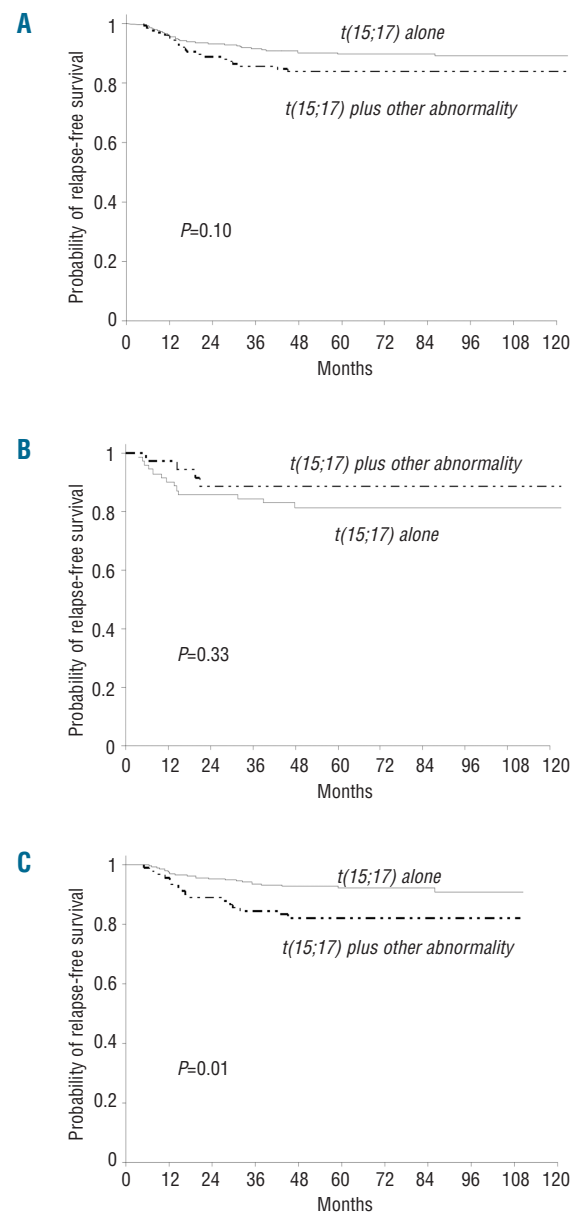
This study shows that roughly one third of patients with APL have ACA besides the  $t(15;17)$ . Among these secondary chromosome aberrations, trisomy 8 is by far the most frequent abnormality, accounting for about one third of the additional abnormalities. In the context of state-of-the-art treatment based on a combination of ATRA and anthracycline-based chemotherapy, the presence of ACA, particularly two or more, or of trisomy 8 was associated with lower platelet counts, a higher relapse-risk score and lower relapse-free survival. However, multivariate analysis showed that neither the presence of ACA nor trisomy 8 is an independent adverse factor for relapse.

The incidence of ACA in APL has been consistently reported to be within the range of 26% to 39%,<sup>1,4,8,23</sup> trisomy 8 being the most frequent abnormality (33% to 53% of secondary changes). The incidence of ACA and the proportion of trisomy 8 among these abnormalities reported in the present study, 28% and 36%, respectively, are both within the ranges reported in the literature. It should be noted that the prevalence of  $abn(7q)$ , the most common abnormality after trisomy 8, is usually in the range from 5% to 8%,<sup>4,6,24</sup> and in our study was 5%, but in a recent study by the German Acute Myeloid Leukemia Study Group (AMLSG) the prevalence was much higher (27% of aberrations).<sup>8</sup> This German study was, however, based on a small series of seven patients displaying this abnormality among only 26 patients with additional changes.

The relative high frequency of some additional chromosome abnormalities, particularly trisomy 8, may suggest the appropriateness of performing a systematic FISH analysis including a centromeric probe for chromosome 8 in the diagnostic work-up of patients with APL and perhaps extending this to the detection of  $del(7q)$ . As has been previously reported,<sup>14</sup> we found that patients with  $t(15;17)$  not detected by conventional karyotyping had the same pattern of ACA as patients with conventionally identified  $t(15;17)$ , with chromosome 8 abnormalities being most common. This finding would suggest that the ACA are important cooperating lesions in the leukemogenesis of APL.

With regards to clinicopathological characteristics, the association of ACA with low platelet counts, intermediate- and high-risk disease, and the presence of coagulopathy found in the present study has not been previously reported as far as we know. At the molecular level, a previous study found a relationship between the breakpoint at the BCR3 region and the presence of ACA.<sup>4</sup> We did not demonstrate a statistically significant relation between the

BCR3 isoform and the presence of ACA, but there was a tendency for the two to be associated ( $P=0.08$ ). Another interesting relationship, between ACA and the mutational status of the *FLT3* gene, has been recently suggested.<sup>24,25</sup> A Medical Research Council study<sup>24</sup> revealed an inverse relationship between the frequency of *FLT3*/TTD and presence of ACA accompanying  $t(15;17)$  analyzed by conventional cytogenetics. This finding has also been reported by Akagi *et al.*<sup>25</sup> who analyzed ACA with high-density single-nucleotide polymorphism microarray to detect copy-number-neutral loss of heterozygosity. Interestingly, *FLT3*/TTD mutations occurred only in the group with no genomic alterations. In our series, this mutation occurred in a lower



**Figure 1.** Relapse-free survival of patients according to the presence of additional chromosomal abnormalities in the: (A) LPA96 and LPA99 trials, (B) LPA96 trial, and (C) LPA99 trial.

proportion of patients with trisomy 8 (11.5%) than in those with other ACA (24.3%) or without ACA (22.8%), but the differences were not statistically significant.

Although the AMLSG study<sup>8</sup> reported that patients dying during induction therapy had significantly higher initial white blood cell counts and a higher likelihood of trisomy 8 or abn(7q) as ACA, no other study has found such an association between cytogenetics and induction outcome. Indeed, we found that patients with ACA had a similar induction death rate as those with only the t(15;17). The association observed in the present study between ACA and coagulopathy, which is potentially implicated in an increased risk of induction death,<sup>18</sup> could explain in part the results of the German study.

As far as we know, only two contradictory studies based on small series of patients treated in the pre-ATRA era have previously shown some association between ACA and relapse.<sup>4,5</sup> In 54 patients (44 treated without ATRA), Hiorns *et al.*<sup>5</sup> found that relapse-free survival was significantly correlated with karyotype: patients without ACA and with a low white blood cell count had a significant advantage in terms of relapse-free survival in comparison with patients with other combinations of these factors. In contrast, Slack *et al.*<sup>4</sup> in a study carried out in 80 patients treated with chemotherapy alone, found that the presence of a secondary chromosome abnormality was associated with a longer complete remission duration. Our study, based on a large series of patients treated with ATRA plus anthracycline-based chemotherapy with prolonged follow-up, does not confirm a significant relapse-free survival disadvantage in APL patients with ACA. It should be noted that the adverse prognostic impact of ACA on relapse observed in the LPA99 trial, which was not independent of relapse-risk score, male gender, and BCR3 isoform, was not observed in the LPA96 trial. Apart from differences in sample size that could explain a different impact of ACA in the LPA96 and LPA99 trials, it is well known that the efficacy of therapy can have a critical influence on the prognostic significance of other variables. In this regard, previous reports described a lower relapse-free survival in the LPA99 trial,<sup>10,11</sup> which may have contributed to altering the prognostic value of many variables, including ACA. We can also speculate that the adverse impact of ACA on relapse-free survival in the LPA99 trial, which was revealed by univariate analysis, could be masked in multivariate analysis because of the association of such abnormalities with intermediate- and high-risk groups and the BCR3 isoform. It is conceivable that ACA in general, or some specific abnormality in particular (e.g., trisomy 8), might have a role in generating the factors leading to a poorer risk score. Further studies to confirm and elucidate the relative importance of these variables are warranted.

In conclusion, this study confirms that one third of patients with *de novo* APL display ACA at diagnosis, trisomy 8 being the most frequent abnormality. Patients with ACA had significantly more coagulopathy, and were less frequently classified as being at a low-risk of relapse. Although ACA and trisomy 8 were significantly associated with lower relapse-free survival, they were not identified as independent risk factors for relapse, probably because of their association with relapse-risk score. Until confirmation of this hypothesis, additional therapeutic strategies are

not required in APL patients with ACA, at least in the context of ATRA plus anthracycline monotherapy-based regimens.

## Authorship and Disclosures

JC, PM, and MS conceived the study, and analyzed and interpreted the data; JC, PM, MS, and BL wrote the paper; PM performed the statistical analyses; JC, JMH, MJC, AA, MTF, EL, and JS were responsible for the main cytogenetic laboratories; EV, CR, GM, JS, CR, JDG, MT, EA, MG and SB were clinicians responsible for the patients: they took care of the protocol, sampling and collection of clinical data for the patients treated in their institutions.

All authors reviewed the manuscript and contributed to the final draft.

The authors declare they have no conflicts of interest.

## Appendix

The following institutions and clinicians participated in the study: Argentina (Grupo Argentino de Tratamiento de la Leucemia Aguda)—Complejo Médico Policía Federal, La Plata: L. Palmer; Fundaleu, Buenos Aires: S. Pavlovsky, G. Milone, I. Fernández; Hospital Clemente Álvarez, Rosario: S. Ciarlo, F. Bezares; Hospital de Clínicas, Buenos Aires: H. Longoni; Hospital General San Martín, La Plata: M. Gelemur, P. Fazio; Hospital Rossi, La Plata: C. Canepa, S. Saba; Hospital San Martín de Paraná, Entre Ríos: P. Negri; Instituto Privado de Hematología, Paraná: M. Giunta; Instituto de Trasplante de Médula Ósea, La Plata: J. Milone, V. Prates; Czech Republic—Faculty Hospital, Brno: M. Protivankova; Spain (Programa Español de Tratamiento de las Hemopatías Malignas)—Basurto Hospital, Bilbao: J. M. Beltrán de Heredia; Complejo Hospitalario de Segovia: J. M. Hernández; Complejo Hospitalario Xeral-Calde, Lugo: J. Arias; Complejo Hospitalario, León: F. Ramos; Fundación Jiménez Díaz, Madrid: A. Román; Hospital 12 de Octubre, Madrid: J. de la Serna; Hospital Carlos Haya, Málaga: S. Negri; Hospital Central de Asturias, Oviedo: C. Rayón; Hospital Clinic, Barcelona: J. Esteve; Hospital Clínico de Valladolid: F.J. Fernández-Calvo; Hospital Clínico San Carlos, Madrid: J. Díaz Mediavilla; Hospital Clínico San Carlos (H. Infantil), Madrid: C. Gil; Hospital Clínico Universitario, Santiago de Compostela: M. Pérez; Hospital Clínico Universitario, Valencia: M. Tormo; Hospital Clínico Universitario Lozano Blesa, Zaragoza: M. Olave; Hospital de Cruces, Baracaldo: E. Amutio; Hospital del Mar, Barcelona: C. Pedro; Hospital de Navarra, Pamplona: A. Gorosquieta; Hospital Dr Negrín, Las Palmas: T. Molero; Hospital Dr Peset, Valencia: M. J. Sayas; Hospital Dr Trueta, Girona: R. Guardia; Hospital General de Albacete: J. R. Romero; Hospital General de Alicante: C. Rivas; Hospital General de Alicante (Oncología Pediátrica): C. Esquembre; Hospital General de Castellón: R. García; Hospital General de Especialidades Ciudad de Jaén: A. Alcalá; Hospital General de Jerez de la Frontera: A. León; Hospital General de Murcia: M.L. Amigo; Hospital General de Valencia: M. Linares; Hospital Germans Trias i Pujol, Badalona: J. M. Ribera; Hospital Insular de Las Palmas: J. D. González San Miguel; Hospital Juan Canalejo, A Coruña: G. Debén; Hospital Joan XXIII, Tarragona: L. Escoda; Hospital La Princesa, Madrid: R. de la Cámara; Hospital Materno-Infantil de Las Palmas: A. Molines; Hospital do Meixoeiro, Vigo: C. Loureiro; Hospital Montecelo, Pontevedra: M. J. Allegue; Hospital Mutua de Terrasa: J. M. Martí; Hospital Niño Jesús, Madrid: L. Madero; Hospital Ntra. Sra. de Sonsoles, Ávila: M. Cabezu; Hospital Ramón y Cajal, Madrid: J. García-Laraña; Hospital Reina Sofía, Córdoba: R. Rojas; Hospital Río Carrión, Palencia: F. Ortega; Hospital Río Hortega, Valladolid: M. J. Peñarrubia; Hospital San Jorge, Huesca: F. Puente; Hospital San Rafael, Madrid: B. López-Ibor; Hospital Sant Pau, Barcelona: S. Brunet; Hospital San Pedro de Alcántara, Cáceres: J. M. Bergua; Hospital Santa María del Rosell, Cartagena: J. Ibáñez; Hospital Severo Ochoa, Leganés: P. Sánchez; Hospital Son Dureta, Palma de Mallorca: A. Novo;



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